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SPECTRAL ANALYSIS OF METHANOLIC ROOT BARK EXTRACT OF SEMAL (*BOMBAX CEIBA*) USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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ABSTRACT: *Bombax ceiba* L. is an important medicinal plant belonging to the family Bombacaceae. Root bark samples of the plant have been subjected to phytochemical investigation through gas chromatography-mass spectrometry (GC-MS). Twenty phytochemical compounds have been identified in the methanolic extract of *B. ceiba* root bark. The identification of phytochemical compounds is based on the peak area, retention time, molecular weight and molecular formula.

INTRODUCTION: *Bombax ceiba* L. of family Bombacaceae which contain about 26 genera and nearly 140 pantropical species [1]. It is commonly known as semal or silk cotton tree or Indian kapok [2]. *B. ceiba* is widely distributed throughout Nepal, India, West China, Malaysia, and the hotter part of India [3]. Semal is a lofty, deciduous tree that is up to 40 m tall and 6 m in girth with horizontally spreading branches and a young stem covered with stout, hard prickles. The bark is pale ash to silver-grey in color. Flowers are large in diameter, red in colour and numerous with copions nectar. The fruits are brown capsule-like up to 15 mm long, filled with numerous black seeds which are irregular obovoid in shape, smooth and oily with dense silky hair. The fruit pulp is sweet and edible. Semal tree have compound leaves which are palmate in appearance.

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It is digitate, large, spreading, glabrous, which has common petioles, and the size of the leaf is 15-30 cm long. The size of leaflets varies from 10 cm to 20 cm. new leaves usually do not appear until flowering is over. The bark of this plant is used in wound healing, flower and fruits are used in revitalizing sexual impotency and gum are used as a remedy of diarrhea, dysentery, influenza, menorrhagia, and disease of CNS[4]. Methanolic extract of bark of *B. ceiba* showed α-glucosidase inhibitory activity. Cadinane sesquiterpenoids isolated from *B. ceiba* root bark were found to possess hypoglycaemic activity [5], antifungal [6], cytotoxic [7], anti-HIV activity [8]. It is used as folk medicine because of its demulcent, diuretics, restorative, aphrodisiac, and emetic properties [9-11].

Root bark sample of the plant was subjected to phytochemical investigation through Gas Chromatography-Mass Spectrometry (GC-MS). *B.ceiba* is an important medicinal plant; the phytochemical study revealed that it is rich in phenolic compounds while mangiferin a xanthone is present in large amounts in the leaves and obtained directly from the extract.

It has a significant effect as a hypotensive agent and possesses hypoglycemic activity with negligible toxicity. Twenty phytochemicals compounds were identified in the methanolic extract of *B. ceiba* root bark **Table 1**. The identification of phytochemical compounds is based on the peak area, retention time, molecular weight and molecular formula. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of *B. ceiba* root bark **Fig. 1** revealed the existence of lupeol, squalene, lup-20(29)-ene-3-ol, palmitic acid, vitamin E, alpha tocospiro B, *etc* **Table 2.**

MATERIALS AND METHODS:

Plant Material: Samples of *B. ceiba* root bark were collected from the nursery of the University of Rajasthan, Jaipur city Rajasthan (India). The plant was identified by the department of botany, University of Rajasthan, Jaipur. The root bark was thoroughly washed using deionized water and was transferred to our laboratory. The root bark sample was washed, cleaned, and dried. *B. ceiba* root bark is also packed in sealed polythene bags for further experimental purposes.

Extraction: The shade dried root bark (1 Kg) was finely powdered using a grinder. Then the powdered root bark (approximately 500 gm) was placed in a Soxhlet apparatus and then extracted with 2 L Methanol for 20 h on a water bath. Excess solvent was removed under vacuum in a rotary evaporator (Rotary Vacuum Evaporator N. N. Series equipped with an aspirator and a digital water bath SB-651; Eyela, Tokyo, Japan) at 45 °C and further made moisture free with sodium sulphate. The resulting extract was stored at 4 °C until further analysis.

GC-MS Analysis: Gas Chromatography combined with Mass Spectrometry is a preferred methodology for routine analysis of compounds. The GC-MS analysis of the above-mentioned extracts was performed with a Gas Chromatography unit Shimadzu GCMS-QP2010 Plus comprising AOC-20i+s autosampler. Various components were identified by different retention times, which were detected by a mass spectrophotometer. The chromatogram plot of intensity against retention time was recorded by the software attached to it. From the graph, the compounds are identified, comparing the data with the existing software libraries like WILEY8.lib, NIST11.lib, NIST11s.lib, FFNSC2.lib and mass spectra of standard. The name, molecular weight, and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION: The GC spectrum of the methanolic extract shows total 20 compounds present in the methanol extract were determined by the chromatographic method with the help of NIST and WILLEY library, as shown in **Table 1**. Compound Lupeol was found to be in the highest concentration (23.08%) followed by squalene (8.34%), lup-20(29)-en-3-ol (7.67%), and palmitic acid (5.70%) **Scheme I**. Other compounds were found in trace amount **Table 1.** Either one or all the identified compounds may be responsible for the biological activity of the methanolic extract. Further separation of the identified compounds will be done in due course.

Lupeol (1), a phytoconstituent belonging to the triterpene class, is found in several fruit plants and medicinal plants that have been the object of study in the treatment of various diseases, including skin wounds. Various medicinal properties of lupeol have been reported in the literature, including anti-inflammatory, antioxidant, anti-diabetic, and anti-mutagenic effects. Investigation effects of lupeol (0.1, 1, 10 and 20 µg/mL) on *in-vitro* wound healing assays and signaling mechanisms in human neonatal foreskin keratinocytes and fibroblasts showed that, at high concentrations, Lupeol reduced cell proliferation of both keratinocytes and fibroblasts, but increased *in-vitro* wound healing in keratinocytes and promoted the contraction of dermal fibroblasts in the collagen gel matrix.

This triterpene positively regulated matrix metalloproteinase (MMP)-2 and inhibited the NF-κB expression in keratinocytes, suggesting an anti-inflammatory effect. Lupeol also modulated the expression of keratin 16 according to the concentration tested. Additionally, in keratinocytes, lupeol treatment resulted in the activation of Akt, p38, and Tie-2, signaling proteins involved in cell proliferation and migration, angiogenesis, and tissue repair. These findings suggest that lupeol has therapeutic potential for accelerating wound healing.

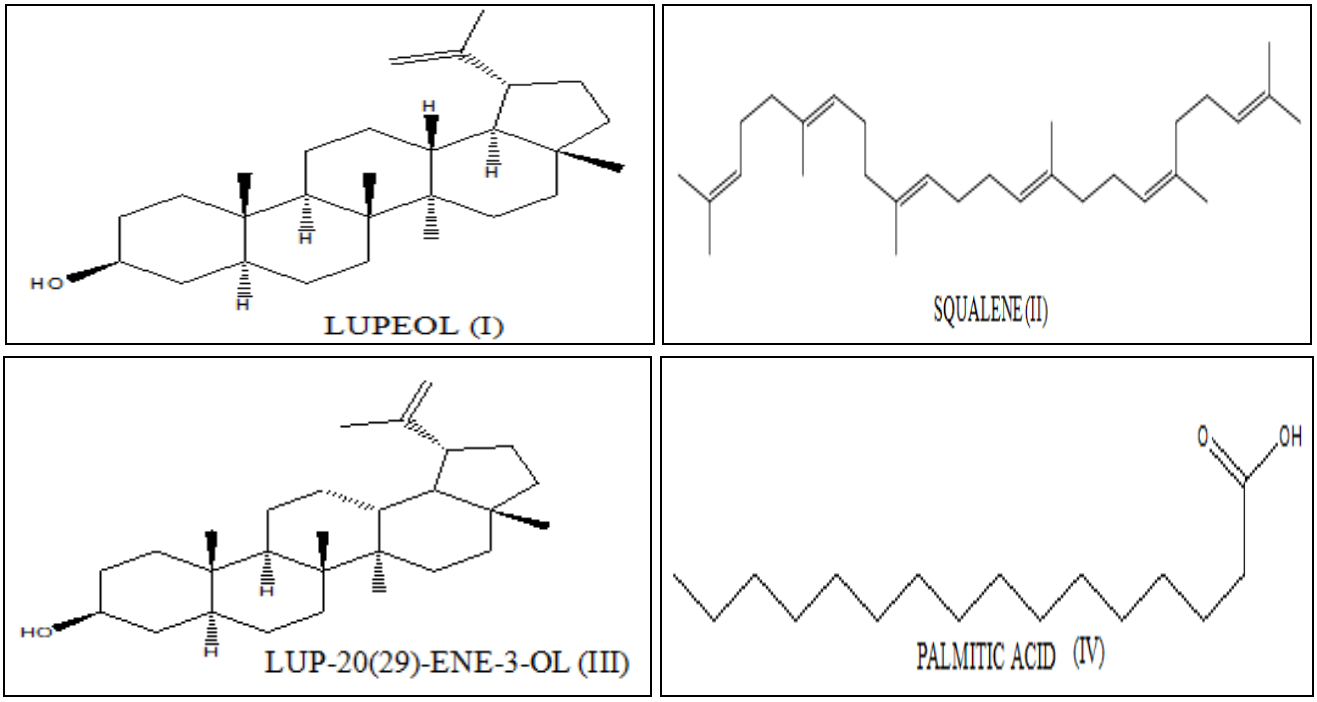

TABLE 1: PHYTOCHEMICALS IDENTIFIED IN THE METHANOL EXTRACT OF THE ROOT BARK OF *B. CEIBA* L. BY GC-MS

Peak #	R. time	Area	Area%	Name
1	10.657	1574098	4.18	5-hydroxymethylfurfural
2	11.382	202934	0.54	2-methoxy4-vinyl phenol
3	17.172	457271	1.24	Plichidiol
4	17.421	428821	1.14	Neophytadiene
5	18.334	1207434	3.20	Hexadecanoic acid methyl ester
6	18.770	2148247	5.70	Palmitic acid
7	19.000	290532	0.77	Hexadecanoic acid ethyl ester
8	19.956	629750	1.67	(z,z)-9,12,-octadecadinoic acid
9	20.015	944697	2.51	8,11,14-docosatrienoic acid
10	20.142	374048	0.99	Phytol
11	20.255	292162	0.78	Methyl strearate
12	20.440	1017163	2.70	(z)-7-tetradecenal
13	20.636	489206	1.30	9-octadecenoic acid phytol acetate
14	21.025	43447715	1.15	Phytol acetate
15	25.819	3142777	8.34	Squalene
16	29.393	1510904	4.01	Vitamin E
17	32.74217	361045	0.96	Gamma sitosterol
18	33.892	2891926	7.67	Lup-20(29)-en-3-ol
19	34.628	8698851	23.08	Lupeol
20	36.834	1405318	3.73	Lup-20(29)-en-3-one

TABLE 2: MAJOR PHYTOCHEMICALS IDENTIFIED IN THE METHANOL EXTRACT OF THE ROOT BARK OF *B. CEIBA* L.

S. no.	Phytochemical compound	RT (min)	Molecular Formula	Molecular weight	MS Fragment –ions	Area %
1	Lupeol	34.608	$C_{30}H_{50}O$	426	27,41,55,69,81,95,107 121,135,189,207	23.08
2	Squalene	25.819	$C_{30}H_{50}$	410	41,69,81,95,121,137,149	8.34
3	Lup-20(29)-en-3-ol	33.892	$C_{30}H_{50}O$	424	27,41,55,67,81,95,109,121,135,189,205,313	7.67
4	Palmitic acid	18.770	$C_{15}H_{31}O_2$	256	40,41,43,60,73,85,98,115,129,143,157,171	5.70

Experimental Section: Characterization data. **Compound 1 Lupeol:** White powder; ^{1}H NMR (300 MHz, $CDCl_3$): δH 0.75, 0.78, 0.82, 0.93, 0.95, 1.02, 1.25 (each, 3H, *s*, CH_3 × 7), 3.22 (1H, *dd*, *J* = 6.8 Hz, H-3), 3.20 (1H, *dd*, *J* = 7.6, H-3); 4.58 (3H, *s*, *J* = 2.0 Hz, H - 29), 0.76 (3H, *s*, H-23), 0.79 (3H,

s, H-24), 0.83 (3H, *s*,H-25), 0.86 (3H, *s*, H-26), 0.88 (3H, *s*, H-27), 0.87 (3H, *s*, H-28), 1.68 (2H, *d*, H-30), 1.39 (2H, *d*, H- 1), 1.32 (2H, *m*, H-6), 1.36 (*m*, 2H, H-7), 1.39 (*d*, 1H, H-9), 1.36 (*m*, 2H, H-15), 1.36 (2H, *m*, H-16), 1.39 (2H, *m*, H-12), 1.39 (1H, *d*, H-13), 1.32 (2H, *m*, H-11), 1.42 (2H, *m*, H-22), 1.53 (2H, *m*, H-21), 1.59 (2H, *m*, H-2), 4.58 (3H, *s*,H-29), 1.36 (1H, *d* H-5), 1.42 (, 1H, *d* H-18), 2.37 (1H, *d*, H-19) and 3.18 (1H, *d*, H-3); ^{13}C NMR (300 MHz, $CDCl_3$): δC 19.2 (C- 23),19.3 (C-24), 29.1 (C-25), 20.9 (C-26), 27.4 (C-27), 25.1 (C-28), 109.3 (C-30), 35.2 (C-1), 19.2(C-6), 35.5 (C-7), 50.4 (C-9), 27.4 (C-15), 40.0 (C-16), 34.2 (C-12), 40.0 (C-13), 29.83 (C-11), 42.81 (C-22), 43.0 (C-21), 34.2 (C-2), 29.1 (C- 29), 50.4 (C-5), 48.2 (C-18), 151.0 (C-20), 55.25 (C-19), 79.0 (C-3).

Compound 2: Squalene: Colourless oil; ^{1}H NMR (400 MHz, $CDCl_3$): δH 1.61-1.69 (3H, *s*, *J* = 5.17 Hz, H-19'; 1H, *m*, *J* = 5.12 Hz, H-3), δ 1.30 (*s*, 3H, H-1), 2.04 (*s*, 3H, H-21), 5.12 (*m*, 1H, H- 3), 1.32 (*m*, 2H, H-4), 2.10 (*m*, 2H, H-5), 1.70 (s, 3H, H-6'), 5.10 (*m*, 1H, H-7), 1.32 (*m*, 2H, H-8), 2.10 (*m*, 2H, H-9), 1.70 (*s*, 3H, H-10'), 5.10 (*m*, 1H, H-11), 2.04 (*m*, 2H, H-12), 1.70 (*m*, 2H, H-13), 5.10 (*m*, 1H, H-14), 1.71 (*s*, 3H, H-15'), 2.10 (*m*, 2H, H-16), 1.62 (*m*, 2H, H-17), 5.10 (*m*, 1H, H-18), 5.17 (*s*, 3H, H-19'), 2.08 (*m*, 2H, H-20), 1.32 (*m*, 2H, H-21), 5.10 (*m*, 1H, H-22), 1.30 (*s*, 3H, H-23') 1.80 (*s*, 3H, H-24). ^{13}C NMR (400 M Hz, $CDCl_3$): δC 16.0 (C-1), 134.8 (C-2), 17.6 (C-2'), 124.2 (C-3), 28.2 (C-4), 39.73 (C-5), 135.0 (C-6), 15.9 (C-6'), 124.2 (C-7), 28.2 (C-8), 39.73 (C-9), 135.0 (C-10), 15.9 (C-10'), 124.2 (C-11), 29.67 (C-12), 29.67 (C-13), 124.28 (C-18), 135.0 (C-19, 39.7 (C-20), 28.2 (C-21), 124.3 (C-22), 134.8 (C-23), 16.0 (C-23), 17.6 (C-24).

Compound 3: Lup-20(29)-en-3-ol: White crystalline powder (needles). M.p. 214–216 °C. IR-FT (KBr): 3550, 3400, 3295, 2920, 2850, 1640 (weak), 1455, 1380, 1040, 1015, 880. ^{1} H-NMR (400 MHz; $CDCl_3$) 4.69 (br. d, 2.5 Hz, H-29b), 4,57 (dt, 2.5, 2.5 e 1.3 Hz, H-29a), 3.19 (dd, 11.0 e 5.0 Hz, H-3), 2.38:δ (dt, 11.0 e 5.8 Hz, H-19), 1.68 (br. dd, 1.3 e 0.8 Hz, H-30), 1.03 (s, H-26), 0.97 (s, H-23), 0.94 (br. d, :δ0.8 Hz, H-27), 0.83 (br. d, 0.8 Hz, H-25), 0.79 (s, H-28), 0.76 (s, H-24). ^{13}C-NMR (100 MHz; $CDCl_3$) 0.96 (C-20), 109.32 (C-29), 79.01 (C-3), 55.32 (C-5), 50.46 (C-9), 48.33 (C-18), 47.78 (C-19), 43.01 (C-17), 42.85 (C-14), 40.85 (C-8), 40.02 (C-22), 38.87 (C-4), 38.73 (C-1), 38.07 (C-13), 37.19 (C-10), 35.60 (C-16), 34.30 (C-7), 29.87 (C-21), 28.00 (C-23), 27.46 (C-15), 27.43 (C-2), 25.17 (C-12), 20.95 (C-11), 19.32 (C-30), 18.33 (C-6), 18.01 (C-28), 16.12 (C-25), 15.99 (C-26), 15.37 (C-24), 14.56 (C-27). MS, m/z (%): 69 (100), 55 (72), 41 (64), 135 (52), 133 (34), 197 (28), 203 (26), 218 (26), 121 (44), 189 (40), 409 (1), 408 (1), 426 (1, M+, $C_{30}H_{50}O$).

Compound 4 Palmitic Acid: White solid; ^{1}H NMR (400 MHz, $CDCl_3$): δH 2.35 (2H, *t*, *J* = 7.6 Hz, H-2), 0.89 (3H, *t*, *J* = 6.8 Hz, H-16), 1.66 (2H, *q*, *J* = 7.6 Hz, H-3), 1.26 (2H, *m*, H-4, H-5, H-6, H-7, H-8, H-9, H-10, H-11, H-12, H-13, 14, 15). ^{13}C NMR (400 MHz, $CDCl_3$): δC 180.1(C-1), 33.9 (C-2), 24.6 (C-3), 29.0 (C- 4), 29.2 (C-5), 29.3 (C-6), 29.4 (C-7), 29.5 (C-8), 29.6 (C-9), 29.6 (C-10), 29.6 (C-11), 31.9 (C-12), 29.6 (C-13), 29.6 (C- 14), 22.7 (C-15), 14.0 (C-16).

CONCLUSION: Present study of the methanol extract of *B. ceiba* root bark indicated that it contains biologically active compounds. The properties of these compounds probably contribute, at least to some extent, to the pharmacological and traditional medicinal uses of the *B. ceiba*. Further separation and identification of compound present in it may give new biologically active compounds, which can be used as lead compounds in the future.

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CONFLICTS OF INTEREST: Nil

REFERENCE:

1. Nandkarni's KM: Indian Materia Medica *Bombay*: Popular Prakashan 1996; 22: 300-302.
2. Krishnamurthi SA and Chadha YR: The wealth of India 1972; 9: 177-83.
3. Krishnamurthi SA and Chadha YR: The Wealth of India 1972; 9: 175.
4. Wu PL, Su GC and Wu TS: Constituents from the Stems of Aristolochia m anshuriensis. J Nat Pr 2003; 66: 996-98.
5. Saleem R, Ahmad M, Hussain SA, Qazi AM, Ahmad SI, Qazi MH, Ali M, Faizi S, Akhtar S and Husnain SN: Hypotensive, hypoglycaemic and toxicological studies on the flavonol C-glycoside shamimin from *Bombax ceiba*, Planta Med 1999; 65: 331-34.
6. Reddy MV, Reddy MK, Gunasekar D, Murthy MM, Caux C and Bodo B: A new sesquiterpene lactone from *Bombax*

ceiba. Chemical and pharmaceutical bulletin. Chem Pharm Bull 2003; 51: 458-59.
7. Shahat AA, Hassan RA, Nazif NM, Miert SV, Peters L, Hammuda MF and Vlietinck AJ: Isolation of mangiferin from *Bombax ceiba* and structure revision of shamimin. Planta Med 2003; 69: 1068-70.
8. Sood RP, Suri KA, Suri OP, Dhar KL and Atal CK: Sesquiterpene lactone from *Salmalia malbarica*. Phytochemistry 1982; 21: 2125-26.
9. Bose S and Dutta AS: Nature of sugars present and the structure of aldobiuronic acid, J Indian Chem Soc 1963; 40: 257-62.
10. Sankaram AVB, Reddy NS and Shoolery JN: New sesquiterpenoids of *Bombax ceiba*. Phytochemistry 1981; 20: 1877-81.
11. Tiew P, Puntumchai A, Kokpol U and Chavasiri W: Coumarins from the heartwoods of *Mansonia gagei* Drumm. Phytochemistry 2002; 6: 773-776.

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